



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| (51) International Patent Classification ⁶ : C08G 69/36, A61K 31/795 | A1 | (11) International Publication Number: WO 98/03572 (43) International Publication Date: 29 January 1998 (29.01.98) |
| (21) International Application Number: PCT/AU97/00446 (22) International Filing Date: 17 July 1997 (17.07.97) (30) Priority Data: PO 1043 17 July 1996 (17.07.96) AU (71) Applicant (for all designated States except US): BIOMOLECULAR RESEARCH INSTITUTE LTD. [AU/AU]; 343 Royal Parade, Parkville, VIC 3052 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): MATTHEWS, Barry, Ross [AU/AU]; 9 Roy Road, Olinda, VIC 3788 (AU). HOLAN, George [AU/AU]; 86 Were Street, Brighton, VIC 3186 (AU). (74) Agents: SLATTERY, John, Michael et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU). | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> |
| (54) Title: ANTIVIRAL LINEAR POLYMERS (57) Abstract <p>An antiviral compound comprises a linear non-carbohydrate polymer having a plurality of side chain groups, wherein at least one of the side chain groups has an anionic- or cationic-containing moiety bonded or linked thereto.</p> | | |

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- 1 -

ANTIVIRAL LINEAR POLYMERS

FIELD OF THE INVENTION

5 This invention relates to antiviral agents, and in particular it relates to polyionic, especially polyanionic linear polymers which have been found to have significant antiviral activity, particularly against human immunodeficiency virus (HIV), respiratory syncytial virus (RSV), human influenza virus A and B, Hepatitis B virus (HBV) and other enveloped viruses.

10 BACKGROUND OF THE INVENTION

 It has been established that certain sulfonated polysaccharide compounds have antiviral activity when screened against HIV, however these compounds are relatively unstable and accordingly large amounts of these compounds are required to obtain effective antiviral
15 effects. In addition, many of these compounds, including heparin and dextran sulfate for example, are potent anticoagulants and because of this activity they are not particularly suited for clinical use as antiviral agents.

 International Patent Application No. PCT/AU95/00350 (WO 95/34595) discloses a
20 class of antiviral compounds comprising a dendrimer such as a polyamidoamine or polylysine dendrimer having a plurality of terminal groups, wherein at least one of the terminal groups has an anionic- or cationic- containing moiety bonded or linked thereto, particularly a sulfonic acid-containing, a carboxylic acid-containing or a trimethylammonium-containing moiety.

25 The present invention provides a new class of antiviral agents based on a particular type of polyionic polymer having a linear "backbone", which have substantial antiviral activity against HIV1 and HIV2, RSV, HBV and human influenza virus A and B. These compounds are well suited for prophylactic and therapeutic use as antiviral agents in humans.

- 2 -

SUMMARY OF THE INVENTION

According to the present invention, there is provided an antiviral compound comprising a linear non-carbohydrate polymer having a plurality of side chain groups wherein
 5 at least one of said side chain groups has an anionic- or cationic-containing moiety bonded or linked thereto.

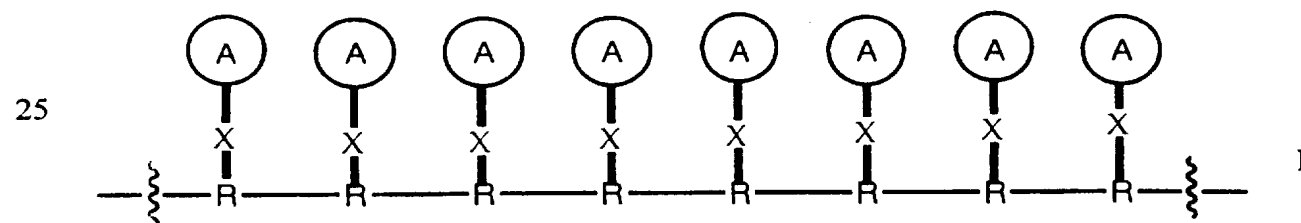
Particularly preferred antiviral compounds of the present invention are linear polymers having sulfonic acid-containing moieties, carboxylic acid-containing moieties, phosphoric or
 10 phosphonic acid-containing moieties, boronic acid-containing moieties, neuraminic or sialic acid-containing moieties or moieties containing neuraminic or sialic acid modified in the 4- or other position thereof, linked to side chain groups thereof.

The compounds of the invention are referred to herein as polyionic polymers, and this
 15 term is used throughout this specification to include not only the polymers *per se*, but also their pharmaceutically or veterinarily acceptable salts, for example the alkaline metal or alkaline earth metal salts such as the sodium, potassium or calcium salts.

DETAILED DESCRIPTION OF THE INVENTION

20

Preferred polyionic polymers in accordance with the present invention are linear polymers having the general formula I:



wherein:

R is a non-carbohydrate monomer unit forming a linear polymer backbone;

30 X is an optional linking group on the side chain groups of monomer units R; and

- 3 -

A is an anionic-containing moiety.

Thus, in accordance with the present invention, the preferred linear polymers are polyanionic materials formed by the conjugation of anionic moieties (A) to a linear non-carbohydrate polymer backbone (made up of a plurality of monomer units R), optionally through linking groups (X). The resultant polyanionic linear polymers have a molecular weight range distribution of repeating units to give a desired median range of molecular weight distribution. Desirably, the median range of molecular weight distribution is from 1,000 to 1,000,000, preferably from 10,000 to 600,000.

10

The monomer unit R is preferably amine or amide moiety, more preferably an amino acid moiety. A particularly preferred monomer unit is a lysine moiety. Poly-L-lysines having various molecular weight ranges are available commercially from Sigma Chemical Company.

15

The anionic moiety A can be linked to reactive side chain groups on the linear polymer backbone either directly or via a variety of functional linking groups X such as, but not limited to, esters, amides, ethers, thioethers, amines, ureas, thioureas, carbamates and carbonates.

20

The optional linking group X may also act as a spacer between the polymer and the anionic moiety A, and may consist of an alkyl chain (optionally substituted or branched), an alkoxy, polyalkoxy, alkylthio or polyalkylthio chain (optionally substituted), or an alkenyl, multiple alkenyl, alkynyl or multiple alkynyl chain (optionally substituted). Suitable spacer chains include groups of the formula $-(CH_2)_n-Z-(CH_2)_n-$, wherein Z is $-CH_2-$, $-CH=CH-$, $-C\equiv C-$, $-O-$ or $-S-$, and n is an integer of from 1 to 15.

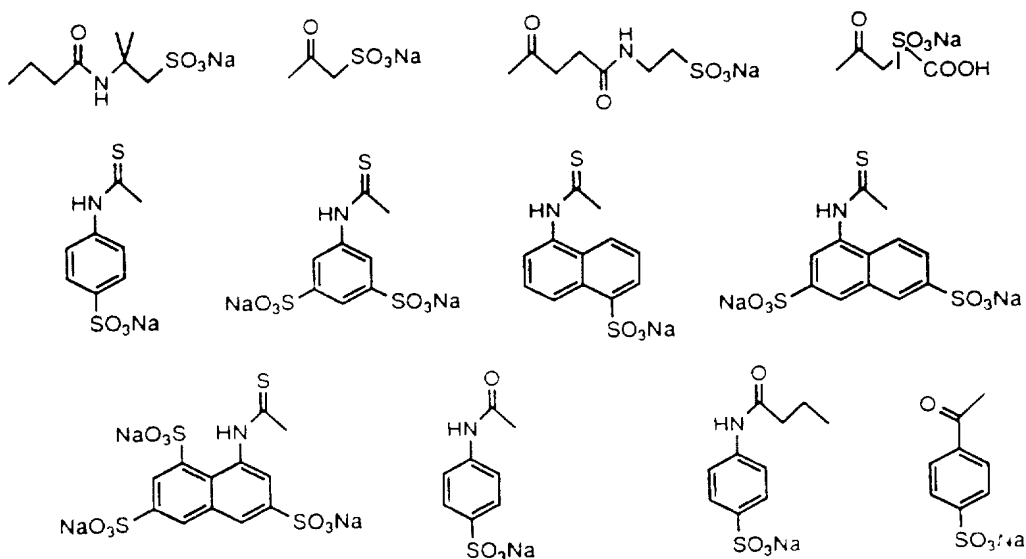
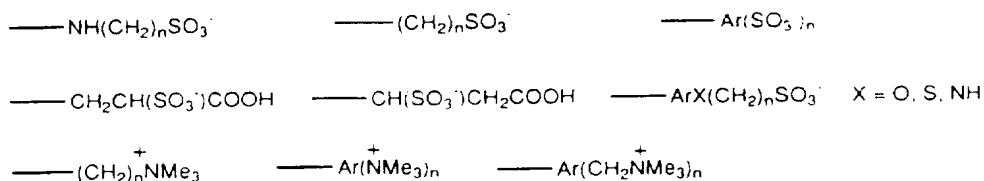
In accordance with the present invention, at least one, and preferably a substantial number, of the reactive side chain groups on the backbone of the linear polymer has an anionic- or cationic-containing moiety covalently bonded thereto. The side chains of the

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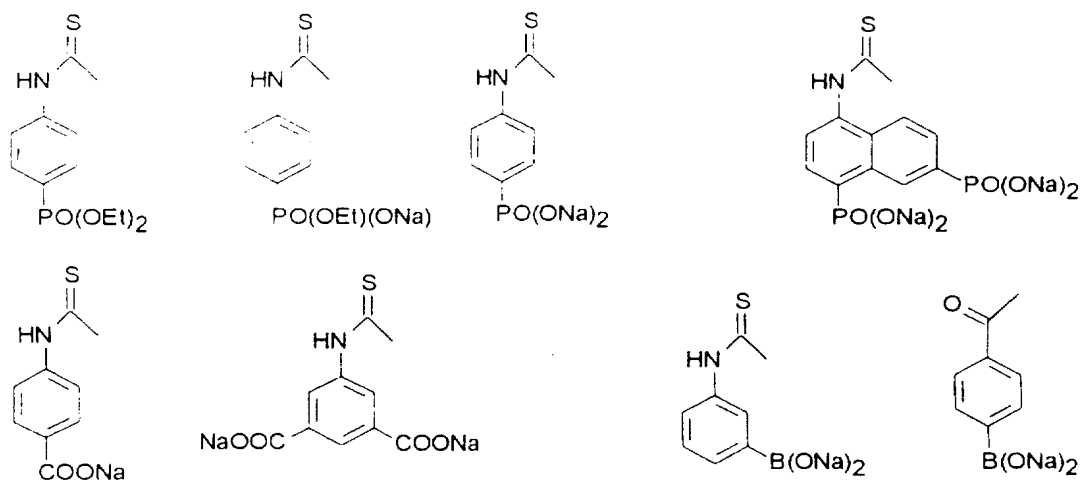
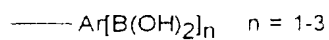
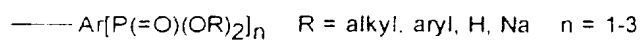
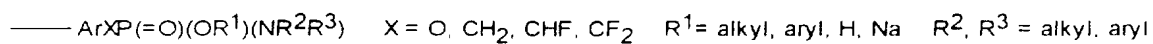
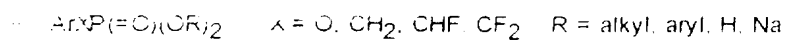
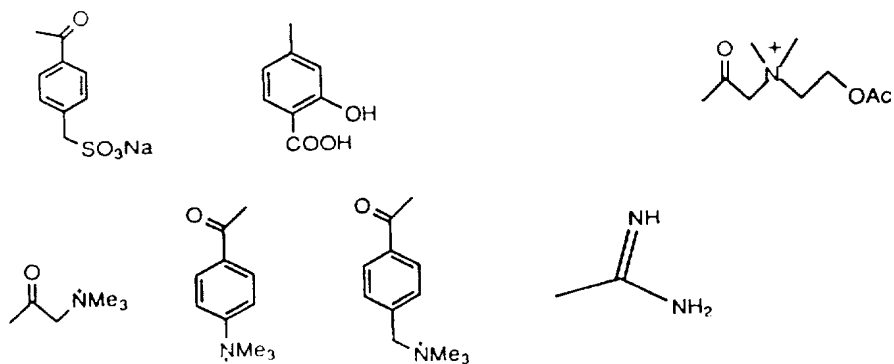
- 4 -

polymer backbone may terminate in amino groups or other functional reactive groups such as OH, SH, or the like, which subsequently can be reacted with the anionic or cationic moieties. Where the side chain groups of the polymer backbone are amine groups, the anionic- or cationic-containing moiety may be linked to the polymer backbone by a variety of functional groups including amide and thiourea linkages. Preferred anionic- or cationic-containing moieties which may be bonded or linked to the side chain groups of the polymer backbone include sulfonic acid-containing moieties, carboxylic acid-containing moieties (including neuraminic and sialic acid-containing moieties and modified neuraminic and sialic acid-containing moieties), boronic acid-containing moieties, phosphoric and phosphonic acid-containing moieties (including esterified phosphoric and phosphonic acid-containing moieties) and trimethylammonium-containing moieties.

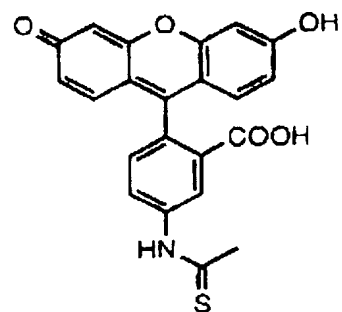
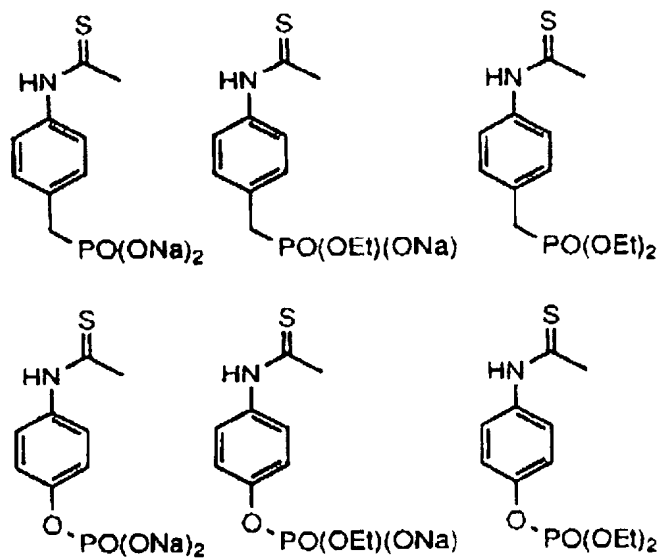
Suitable anionic- and cationic-containing moieties which may be bonded or linked to the amino or other side chain groups of the linear polymers include, by way of example, the following groups (in which n is zero or a positive integer, more particularly n is zero or an integer of from 1 to 20):



- 5 -



- 6 -



- 7 -

In addition to the above, various neuraminic or sialic acid-containing moieties or modified neuraminic or sialic acid-containing moieties may be bonded or linked to the side chain groups in accordance with this invention. These moieties include the various N- and O-substituted derivatives of neuraminic acid, particularly N- and O- acyl derivatives such as N-acetyl, O-acetyl and N-glycolyl derivatives, as well as moieties in which the neuraminic acid group is modified, particularly by substitution in the 4-position, with an amino, amido, cyano, azido or guanidino group.

The polyionic polymers of this invention may be prepared by standard chemical methods which are well known to persons skilled in this art. Suitable methods are described by way of example in the Examples below.

As previously described, the polyionic polymers of the present invention have been found to exhibit significant antiviral activity, particularly against enveloped viruses. Accordingly, these polyionic polymers are useful in prophylactic and therapeutic treatment of viral infections, for example infections by HIV1 and HIV2 and other enveloped viruses including DNA viruses such as Hepatitis B and RNA viruses including flaviviruses such as Hepatitis C, Bovine Viral Diarrhoea Virus and Japanese Encephalitis Virus (JEV). The polyionic polymers of the present invention may also be used in prophylactic or therapeutic treatment against Human Influenza Virus A and B, Rhinoviruses, Corona Viruses, Human Parainfluenza Virus, Respiratory Syncytial Virus (RSV), Varicella Zoster Virus (VZV), Human Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Papilloma Virus (HPV), Adenoviruses, Herpes Simplex Virus (HSV) type 1 and 2, Measles Virus and Vesicular Stomatitis Virus (VSV).

Thus, in another aspect the present invention provides a pharmaceutical or veterinary composition for prophylactic or therapeutic antiviral treatment of a human or non-human animal, which comprises a polyionic polymer as broadly described above, in association with at least one pharmaceutically or veterinarily acceptable carrier or diluent.

- 8 -

The formulation of such compositions is well known to persons skilled in this field. Suitable pharmaceutically acceptable carriers and/or diluents include any and all conventional solvents, dispersion media, fillers, solid carriers, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art, and it is described, by way of example, in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Company, Pennsylvania, USA. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the pharmaceutical compositions of the present invention is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the human subjects to be treated; each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier and/or diluent. The specifications for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active ingredient and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active ingredient for the particular treatment.

In another aspect, the present invention provides a method for prophylactic or therapeutic treatment of a viral infection in a human or non-human animal, which comprises administering to said human or animal a prophylactic- or therapeutic-antiviral-effective amount of a polyionic polymer as broadly described above.

In yet another aspect, this invention provides the use of a prophylactic- or therapeutic-antiviral-effective amount of a polyionic polymer as broadly described above in the prophylactic or therapeutic treatment of, or in the manufacture of a medicament for prophylactic or therapeutic treatment of a viral infection in a human or non-human animal.

- 9 -

A variety of administration routes are available. The particular mode selected will depend, of course, upon the particular condition being treated and the dosage required for therapeutic efficacy. The methods of this invention, generally speaking, may be practised using any mode of administration that is medically acceptable, meaning any mode that
5 produces therapeutic levels of the active component of the invention without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, topical, nasal, inhalation, transdermal or parenteral (e.g. subcutaneous, intramuscular and intravenous) routes. Formulations for oral administration include discrete units such as capsules, tablets, lozenges and the like. Other routes include intrathecal administration
10 directly into spinal fluid, direct introduction such as by various catheter and balloon angioplasty devices well known to those of ordinary skill in the art, and intraparenchymal injection into targeted areas.

The compositions may conveniently be presented in unit dosage form and may be
15 prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing the active component into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active component into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

20

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active component, in liposomes or as a suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, or an emulsion.

25

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active component which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using those suitable dispersing or wetting agents and suspending agents. The sterile injectable
30 preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-

- 10 -

acceptable diluent or solvent, for example as a solution in polyethylene glycol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed
5 including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The active compound may also be formulated for delivery in a system designed to administer the active component intranasally or by inhalation, for example as a finely
10 dispersed aerosol spray containing the active component.

Other delivery systems can include sustained release delivery systems. Preferred sustained release delivery systems are those which can provide for release of the active component of the invention in sustained release pellets or capsules. Many types of sustained
15 release delivery systems are available. These include, but are not limited to: (a) erosional systems in which the active component is contained within a matrix, and (b) diffusional systems in which the active component permeates at a controlled rate through a polymer. In addition, a pump-based hardware delivery system can be used, some of which are adapted for implantation.

20

The active component is administered in prophylactically or therapeutically effective amounts. A prophylactically or therapeutically effective amount means that amount necessary at least partly to attain the desired effect, or to delay the onset of, inhibit the progression of, or halt altogether, the onset or progression of the particular condition being treated. Such
25 amounts will depend, of course, on the particular condition being treated, the severity of the condition and individual patient parameters including age, physical condition, size, weight and concurrent treatment. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical
30 judgement. It will be understood by those of ordinary skill in the art, however, that a lower

- 11 -

dose or tolerable dose may be administered for medical reasons, psychological reasons or for virtually any other reasons.

Generally, daily oral doses of active component will be from about 0.01 mg/kg per day to 1000 mg/kg per day. Small doses (0.01-1 mg) may be administered initially, followed by increasing doses up to about 1000 mg/kg per day. In the event that the response in a subject is insufficient at such doses, even higher doses (or effective higher doses by a different, more localised delivery route) may be employed to the extent patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds.

The active component according to the invention may also be presented for use in the form of veterinary compositions, which may be prepared, for example, by methods that are conventional in the art. Examples of such veterinary compositions include those adapted for:

- (a) oral administration, external application, for example drenches (e.g. aqueous or non-aqueous solutions or suspensions); tablets or boluses; powders, granules or pellets for admixture with feed stuffs; pastes for application to the tongue;
- (b) parenteral administration for example by subcutaneous, intramuscular or intravenous injection, e.g. as a sterile solution or suspension; or (when appropriate) by intramammary injection where a suspension or solution is introduced into the udder via the teat;
- (c) topical application, e.g. as a cream, ointment or spray applied to the skin; or
- (d) intravaginally, e.g. as a pessary, cream or foam.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Further features of the present invention will be apparent from the following Examples

- 12 -

which are included by way of illustration, not limitation of the invention.

EXAMPLE 1

5 Preparation of Sodium 4-Sulfophenylthiourea terminated Polylysines.

A Solid sodium 4-sulfophenylisothiocyanate monohydrate (2.55g; 10mmol) was added to a solution of poly-L-lysine (15-30K) (Sigma Chemical Company) (1.0g) in a mixture of water (20ml) and N,N-dimethyl-N-allylamine buffer (pH 9.5; 15ml). The resulting
10 mixture was heated under nitrogen at 53° C for 3 hours, when a ninhydrin test was negative. The cooled mixture was filtered and the filtrate concentrated to give a grey solid residue. The solid residue was redissolved in water and passed through a column of Amberlite IR 120(Na) and then concentrated. The crude product was purified by gel filtration (Sephadex LH20; water) and freeze dried to give sodium 4-sulfophenylthiourea
15 terminated poly-L-lysine **BRI2995** as a white fluffy solid (1.25g).

B Similarly prepared were sodium 4-sulfophenylthiourea terminated polylysines of molecular weight fraction 1-4K **BRI2994**, 4-15K **BRI2967**, 150-300K **BRI2996**.
20

EXAMPLE 2

Preparation of Sodium 3,6-Disulfonaphthylthiourea terminated Polylysines.

25 Solid sodium 3,6-Disulfonaphthylisothiocyanate (200mg; 0.51mmol) was added to a solution of poly-L-lysine (15-30K) (50mg) in a mixture of water (2ml) and N,N-dimethyl-N-allylamine buffer (pH 9.5; 2ml). The resulting mixture was heated under nitrogen at 53° C for 3 hours, when a ninhydrin test was negative. The cooled mixture was filtered and the filtrate concentrated to give a brownish solid residue. The solid residue was redissolved in water and
30 passed through a column of Amberlite IR 120(Na) and then concentrated. The crude product

- 13 -

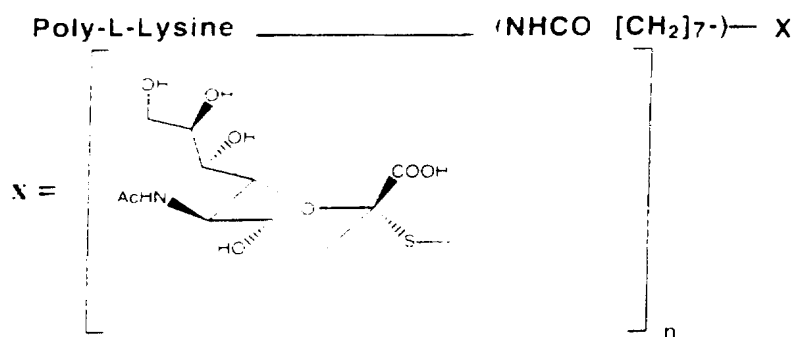
was purified by gel filtration (Sephadex LH20; water) and freeze dried to give sodium 3,6-disulfonaphthylthiourea terminated poly-L-lysine **BRI6047** as a white fluffy solid (87mg).

EXAMPLE 3

5

Preparation of poly-L-lysyl [(8-octanamido)- 5-acetamido-3,5-dideoxy-2-thio-D-glycero- α -D-galacto-2-nonulopyranosidoic acid]_n **BRI 6150**

10



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Methyl [(8-octanoic acid N-hydroxysuccinimide ester) 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero- α -D-galacto-2-nonulopyranosid]onate was prepared by the following procedure. To a solution of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-O-acetyl-3,5-dideoxy-2-thio-D-glycero- α -D-galacto-2-nonulopyranosonate (A. Hasegawa, J. Nakamura, and M. Kiso J. Carbohydrate Chemistry, 5(1), 11-19 1986) (100mg.) in dry dimethylformamide (1ml.) was added 8-bromooctanoic acid (41mg.) and diethylamine (280mg.) and the solution stirred at 20° C for 17 hours. Solvent was removed under vacuum and the residue partitioned between ethyl acetate and ice cold 5% hydrochloric acid. The organic layer was washed with water, dried over sodium sulphate, and evaporated to give a residue (130mg.). This was dissolved in ethyl acetate (5ml.) and N-hydroxysuccinimide (26mg.) and dicyclohexylcarbodiimide (46mg.) were added. The mixture was stirred at 20° C for 17 hours then the white precipitate was filtered off. The filtrate was concentrated and purified by flash chromatography on silica gel eluting with ethyl acetate. Fractions containing

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- 14 -

product were combined and evaporated to give a white foam 97mg. 71%

To a solution of poly-L-lysine.HBr MW 150 - 300Kd (22mg.) in dry dimethyl sulfoxide (1ml.) were added di-isopropylethylamine (15mg.) and methyl [(8-octanoic acid N-hydroxysuccinimide ester) 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero- α -D-galacto-2-nonulopyranosid]onate (90mg.). The mixture was stirred under argon at 20°C for 60 hours then solvent was removed under vacuum. The residue was dissolved in a freshly prepared 0.5M solution of sodium methoxide in methanol (4ml.) and the mixture stirred for 48 hours under argon at 20°C. The solvent was evaporated and the residue dissolved in water (1.5ml.) and stirred for 24 hours . This solution was subjected to size exclusion chromatography on Sephadex LH20 eluting with water. After lyophilisation, the product, poly-L-lysyl[(8-octanamido)-5-acetamido-3,5-dideoxy-2-thio-D-glycero- α -D-galacto-2-nonulopyranosidoic acid]_n was obtained as a white powder 49mg. 94%.

15

EXAMPLE 4

Preparation of Sodium 3,5-Dicarboxyphenylthiourea terminated Polylysines.

A solution of poly-L-lysine.hydrobromide (4-15K0 (Sigma Chemical Company) (50mg) in water (2ml) was added to a solution of sodium 3,5-dicarboxyphenyl isothiocyanate (305mg) in water (3ml) and the pH of the resulting solution adjusted to 9 with aqueous sodium bicarbonate. The solution was then heated at 53°C under nitrogen for 4 hours. The solution was cooled and filtered, and the filtrate concentrated to give an off-white solid residue. The crude product was purified by gel filtration (Sephadex LH20; water) and freeze dried to give sodium 3,5-dicarboxyphenylthiourea terminated poly-L-lysine as a white fluffy solid (71mg).

EXAMPLE 5**Preparation of Sodium 4-(Phosphonomethyl)phenylthiourea terminated Polylysines.**

5 Solid 4-(phosphonomethyl)phenyl isothiocyanate (231mg; 1.0mmol) was added to a solution of poly-L-lysine.hydrobromide (30-70K) (Sigma Chemical Company) (50mg) in a 1:1 mixture of pyridine/water. The pH of the mixture was adjusted to 9.5 with 1M sodium carbonate and the solution heated overnight at 53°C under nitrogen. The mixture was cooled and filtered, and the filtrate concentrated to give a brown solid residue. The crude product
10 was purified by gel filtration (Sephadex LH20; water) to give a brown solid (82mg).

EXAMPLE 6**Preparation of 1-phosphono-oxyphenyl-4-thiourea terminated poly-L-lysine.**

15 To a solution of poly-L-lysine hydrobromide (50mg., Sigma P2636, 30-70 kilodaltons) in water (10ml), heated and stirred at 53°C, was added 4-phosphono-oxyphenyl isothiocyanate (153mg) and the pH of the mixture adjusted to 9.5-10 with 1M sodium carbonate solution. The mixture was heated and stirred at 53°C for 5 hours and then filtered.
20 The clear solution was purified by gel filtration on Sephadex LH20 eluting with water. The eluent was lyophilised to give the product as a white foam. 77mg. 94%.

EXAMPLE 7**25 Preparation of benzamido-4-boronic acid terminated poly-L-lysine.**

To a solution of poly-L-lysine hydrobromide (50mg., Sigma P2636, 30-70 kilodaltons) in DMSO (10ml), under an inert atmosphere, was added 4-carboxyphenylboronic acid N-hydroxysuccinimide ester (90mg) and 1M sodium carbonate solution (2ml) and the mixture
30 stirred at 20°C for 60 hours. Solvent was removed *in vacuo* and the residue dissolved in

- 16 -

water (5ml) and filtered. The clear solution was purified by gel filtration on Sephadex LH20 eluting with water. The eluent was lyophilised to give the product as a white foam. 50mg. 90%.

5

EXAMPLE 8

Test for antiviral activity.

The results of tests of activity of polyionic linear polymers of this invention against
10 HIV 1, human and murine CMV, HSV 1 and 2 and human influenza A are shown in Tables 1 to 4 respectively. Table 5 shows the antiviral activity of the compound of Example 1 against a broad range of viruses. Tables 6 and 7 show the antiviral activity of the compounds of Examples 2 and 3, respectively, against RSV, MV and influenza virus.

15 Using standard test protocols, antiviral activity was measured as an effective dose (EC_{50}) of the test compound that protects at least 50% of the cells used in the test from the effects of the virus. In addition, because of the requirement for safety to the cells by the test compound, cell cytotoxicity (CC_{50}) is determined as the concentration of the test compound that kills 50% of uninfected cells. The total antiviral activity can be expressed as the
20 selectivity index (SI), CC_{50}/EC_{50} .

TABLE 1 Activity of BRI Linear Polymers against HIV

| Compound | Virus Strain | Conc. | EC ₅₀ | CC ₅₀ | Antiviral Index-SI | Structure |
|----------|--------------|-------|------------------|------------------|--------------------|--|
| BRI 6047 | HIV 1 IIIB | μM | 0.002 | >3.44 | >1720 | Poly-L-Lysine(15-30K) Naphthyl di SO ₃ Na |
| BRI 2967 | HIV 1 IIIB | μM | 0.014 | >20.8 | >1480 | Poly-L-Lysine(4-15K) Phenyl-SO ₃ Na |
| BRI 2994 | HIV 1 IIIB | μM | 0.60 | >62.5 | >1040 | Poly-L-Lysine(1-4K) Phenyl-SO ₃ Na |
| BRI 2995 | HIV 1 IIIB | μM | 0.005 | >5.7 | >1140 | Poly-L-Lysine(15-30K) Phenyl-SO ₃ Na |
| BRI 2996 | HIV 1 IIIB | μM | 0.007 | >0.8 | >114 | Poly-d-Lysine(150-300K) Phenyl-SO ₃ Na |

Standards

| | | | | | | |
|------------------------|------------|-------|--------|------|--------|--|
| AZT | HIV 1 IIIB | μg/ml | 0.0001 | 1.17 | 15,775 | |
| DS5000 Dextran Sulfate | HIV 1 IIIB | μg/ml | 0.0263 | >250 | >9,497 | |

TABLE 2 Activity Against Cytomegalovirus (Davis strain) in Cell cultures.

| Compound | EC ₅₀ μg/ml | CC ₅₀ μg/ml | Ganciclovir CC ₅₀ μg/ml | Cells used |
|-----------------------|------------------------|------------------------|------------------------------------|------------|
| BRI 2995 | | | | |
| HCMV CPE Inhibition | 2.7 | >100 | 0.1 | HFF |
| HCMV Plaque Reduction | 9.1 | >100 | 0.2 | HFF |
| MCMV Plaque Reduction | 17.7 | 100 | 0.5 | MEF |

HCMV = Human CytoMegalo Virus; MCMV = Murine (Mouse) CytoMegalo Virus.

Tests in Human Embryonic Lung cells (HEL); Human Foreskin Fibroblasts (HFF); Mouse Embryonic Fibroblasts (MEF).

EC₅₀ CPE = Inhibitory concentration to reduce virus CytoPathic Effect on cells by 50%.

CC₅₀ = Cytotoxic concentration required to reduce HEL cell growth by 50%.

TABLE 3 Activity Against Herpes Simplex 1 & 2 in Cell cultures.

| BRI 2995 | EC ₅₀ µg/ml | CC ₅₀ µg/ml | Ganciclovir CC ₅₀ µg/ml | |
|------------------------|------------------------|------------------------|---------------------------------------|-----|
| HSV-1 CEP Inhibition | 0.88 | >100 | 0.06 | HFF |
| HSV-1 Plaque Reduction | 25.9 | >100 | 0.3 | HFF |
| HSV-2 CPE Inhibition | 1.4 | >100 | 0.2 | HFF |
| HSV-2 Plaque Reduction | 13.6 | >100 | 0.6 | HFF |

HSV-1 = Human Herpes Simplex Virus-1 (Mucosal Herpes).

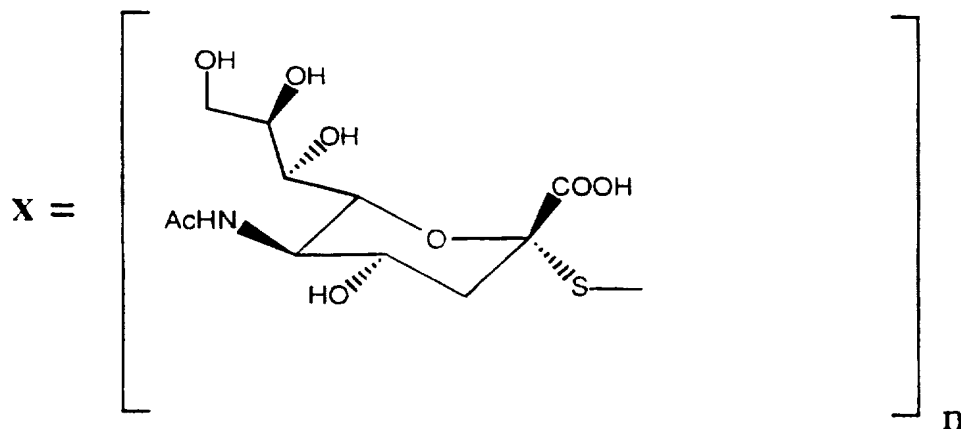
HSV-2 = Human Herpes Simplex Virus-2 (Genital Herpes).

Tests in Human Foreskin Fibroblasts (HFF).

EC₅₀ CPE = Inhibitory concentration to reduce virus CytoPathic Effect on cells by 50%.

EC₅₀ Plaque Reduction = Inhibitory Concentration to reduce virus plaque by 50%.

CC₅₀ = Cytotoxic concentration required to reduce cell growth by 50%.

TABLE 4**BRI 6150****Poly-L-Lysine (590K Average (NHCO [CH₂]₇)- X****Activity Against Two Strains of Influenza A virus**

| Compound | EC ₅₀ µg/ml Tokyo Strain | EC ₅₀ µg/ml G70C Strain |
|----------|--|---------------------------------------|
| BRI 6150 | 0.03 | 0.04 |

TABLE 5 Poly-L-Lysine (NHCSNHPhSO₃Na)x 26-K-53K

| BRI 2995 ARB 95-336 | CPE-inhibition EC ₅₀ (CC ₅₀)μg/ml | | Viral count or Plaque Reduction EC ₅₀ (CC ₅₀) μg/ml | | CPE-inhibition EC ₅₀ (CC ₅₀) μg/ml SI | | Viral Count EC ₅₀ (CC ₅₀) μg/ml SI | |
|--------------------------------------|---|------|--|-------|--|----|---|------|
| Virus; Strain | | | | | | | | |
| | | | | | Ribavirin | | | |
| RSV; Long | 10 (>1000) | >100 | 10 (100) | 10 | 10 (560) | 56 | 200 (200) | 1 |
| | 0.3 (30) | 100 | >0.1 (30) | >300 | 7 (90) | 13 | 1 (40) | 40 |
| RSV; A2 | 0.5 (55) | 110 | 0.1 (>100) | >1000 | 13 (400) | 31 | 2 (>1000) | >500 |
| | | | | | Ganciclovir | | | |
| CMV; Davis | 2.7 (>100) | >37 | 9.1 (>100) | >11 | 0.1 | | 0.2 | |
| MCMV | | | 17.7 (>100) >5.7 | | | | 0.6 | |
| | | | | | Acyclovir | | | |
| VZV | | | 93.3 (>100) >1.1 | | | | 0.5 | |
| HSV-1 | 0.88 (>100) | >125 | >100(>100) 0 | | 0.06 | | 0.3 | |
| HSV-2 | 1.4 (>100) | >71 | >100(>100) 0 | | 0.2 | | 0.6 | |
| | | | | | Control drug (?) | | | |
| Flu A; H ₁ N ₁ | 2.0 (538) | 269 | 2.4 (261) | 109 | 6.1 (>100) >16 | | 5.6 (>100) >18 | |
| Flu B | 6.5 (501) | 77 | 6.5 (171) | 28 | 3.3 (100) >30 | | 6.2 (>100) >16 | |
| | Concentrations μM | | | | Zidovudine | | | |
| HIV-1; IIIB | 0.15 (>250) >1,680 | | | | 0.00037 (4.38) 11,840 | | | |
| HIV-2; ROD | 2.32 (>250) >108 | | | | | | | |

Viruses:

RSV = Respiratory Syncytial Virus;

HSV = Herpes Simplex Virus (type 1 = mucosal; type 2 = genital)

CMV = Cytomegalovirus;

VZV = Varicella Zoster Virus;

MCMV = Murine Cytomegalovirus

Flu A = Influenza A Virus;

Flu B = Influenza B Virus;

HIV-1 & 2 = Human Immunodeficiency Virus type 1 and 2.

Terms:

CPE = Cytopathic effect of virus on infected cells or when measure of cell toxicity (CC₅₀) the effect of compound alone on the cell.

- 20 -

**TABLE 6 Poly-L-lysine CONH.CH₂CH₂NHCSNH[Naphth-3,6-(SO₃Na)₂]
(37K-75K)**

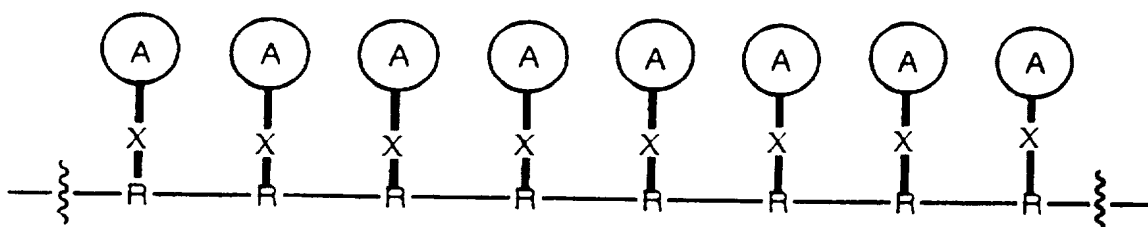
| | | | | |
|-------------------------------|---|---|---|---|
| BRI 6047 ARB-96-222 | CPE-inhibition EC ₅₀ (CC ₅₀) µg/ml SI | PR=Plaque Reduction NR = Neutral Red cell count EC ₅₀ (CC ₅₀) µg/ml SI | CPE-inhibition EC ₅₀ (CC ₅₀) µg/ml SI | PR = Plaque Reduction NR = Neutral Red cell count EC ₅₀ (CC ₅₀) µg/ml SI |
| Virus; Strain | | | Control Drugs | |
| | | | Ribavirin | |
| RSV; A2 | 1 (110) 110 | NR <1.0 (100) >100 | 7 (90) 13 | NR 1.0 (40) 40 |
| RSV; A2 | Virus Yield EC ₅₀ (CC ₅₀) | EC ₉₀ µM SI=CC ₅₀ /EC ₉₀ | Virus Yield EC ₅₀ CC ₅₀ | EC ₉₀ SI=CC ₅₀ /EC ₉₀ |
| RSV; A2 | 1.0 (34) | 3.0 11 | 6.0 (71) | 6.0 12 |
| MV | 18 (55) 3 | | 25 (118) 5 | |
| | | | Control Drug (?) | |
| Flu A; H1N1 | <1.0 (599) >599 | NR <1.0 (178) >178 | 5.6 (>100) >18 | 5.0 (>100) >20 |
| Flu A; H1N1 | 15 (>100) >6.7 | NR 14 (>100) 7.1 | 7.6 (>100) >16 | NR 5.8 (>100) >13 |
| Flu A; H1N1 | 3.8 (>100) 26 | NR 12 (>100) >8.3 | 5.0 (>100) >20 | NR 5.4 (>100) >18 |
| Flu A; H1N1 | 3.2 (>100) >57 | NR 7.2 (>100) >14 | 2.2 (>100) >45 | NR 2.8 (>100) >36 |
| Flu A; H1N1 | Virus Yield EC ₅₀ (CC ₅₀) | EC ₉₀ SI=CC ₅₀ /EC ₉₀ | Virus Yield EC ₅₀ CC ₅₀ | EC ₉₀ SI=CC ₅₀ /EC ₉₀ |
| | 0.53 (140) | 0.8 175 | 4.7 (24) | 3.0 8 |
| Flu B | 13 (487) 37 | NR 19 (267) 14 | 4.3 (100) >23 | 5.5 (>100) >18 |
| Flu B | Virus Yield EC ₅₀ (CC ₅₀) | EC ₉₀ SI=CC ₅₀ /EC ₉₀ | Virus Yield EC ₅₀ CC ₅₀ | EC ₉₀ SI=CC ₅₀ /EC ₉₀ |
| | 2.2 (140) | 49 2.8 | 1.7 (24) | 1.5 16 |

TABLE 7 Poly-L-lysine-{NH.CO.(CH₂)₇S-[2-5-acetyl-neuraminic acid]}_x (590k)

| BRI 6150 ARB-96-227 | CPE-inhibition EC ₅₀ (CC ₅₀) μg/ml SI | CPE-inhibition EC ₅₀ (CC ₅₀) μg/ml SI | CPE-inhibition EC ₅₀ (CC ₅₀) μg/ml SI | CPE-inhibition EC ₅₀ (CC ₅₀) μg/ml SI |
|-------------------------------|---|---|---|---|
| Virus; Strain | | | Control Drug | |
| | | | Ribavirin | |
| RSV; A2 | | <1.0 (>1000) > 1000 | 13.0 (400) 31 | |
| RSV; A2 | | <1.0 (>1000) > 1000 | | 2.0 (>1000) > 500 |
| Flu B | <1 (>720) > 720 | NR <1 >1000 > 1000 | NR 1.8 (>100) > 56 | |
| RSV; A2 | Viral Yield EC ₅₀ (CC ₅₀) | EC ₉₀ SI=CC ₅₀ /EC ₉₀ | Viral Yield EC ₅₀ (CC ₅₀) | EC ₉₀ SI=CC ₅₀ /EC ₉₀ |
| | 3.0 (>1000) | 2.0 > 500 | 6.0 (71) | 6.0 12 |

CLAIMS:

1. An antiviral compound comprising a linear non-carbohydrate polymer having a plurality of side chain groups, wherein at least one of said side chain groups has an anionic- or cationic-containing moiety bonded or linked thereto.
2. A compound according to claim 1, comprising a linear polymer of the general formula I:



wherein:

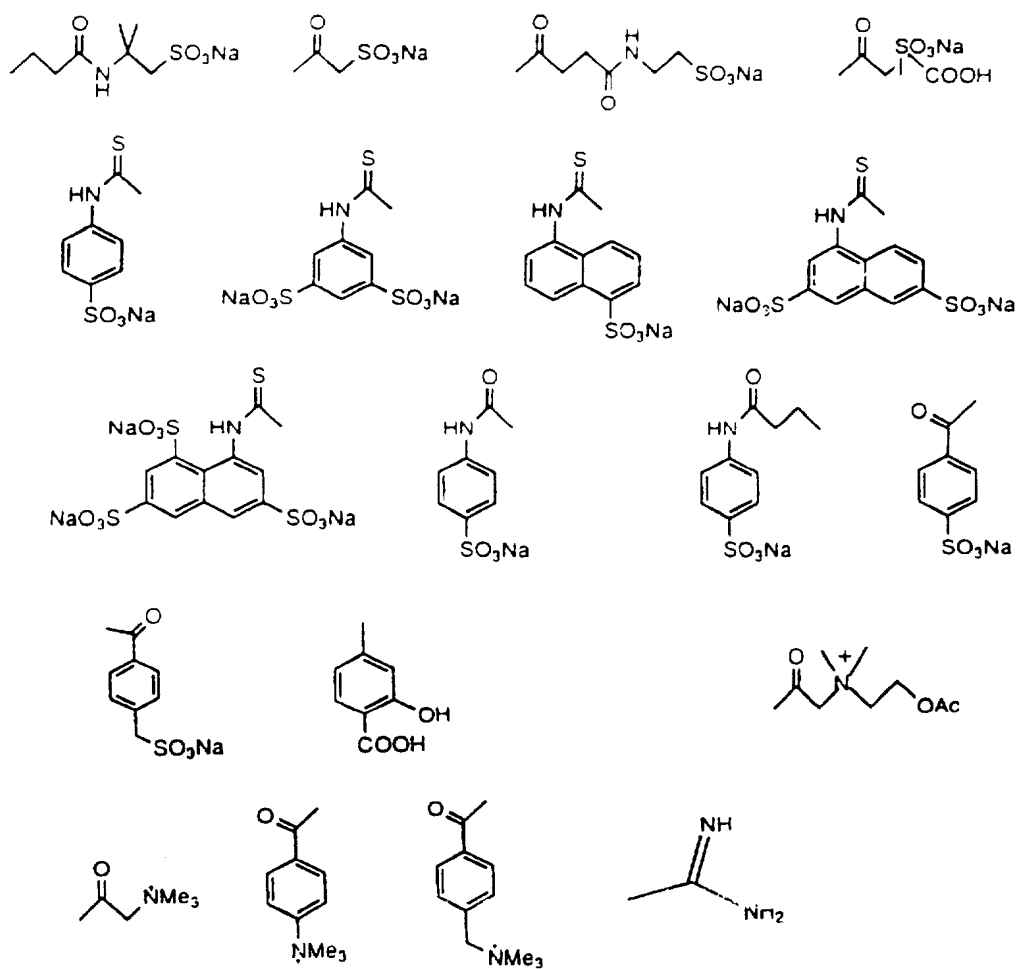
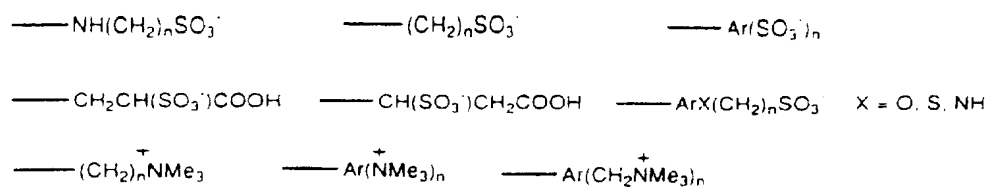
R is a non-carbohydrate monomer unit forming a linear polymer backbone;

X is an optional linking group on the side chain groups of monomer units R; and

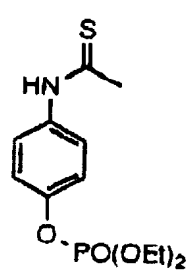
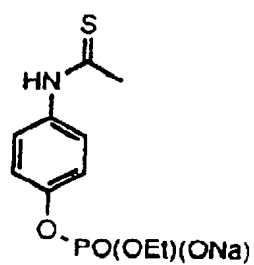
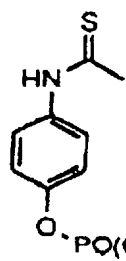
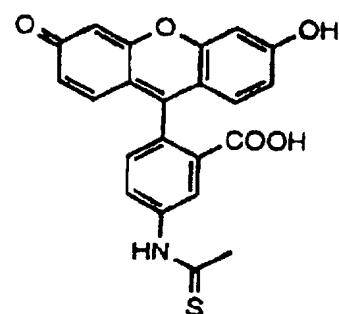
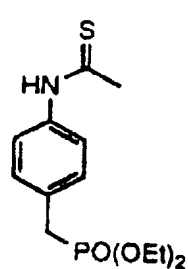
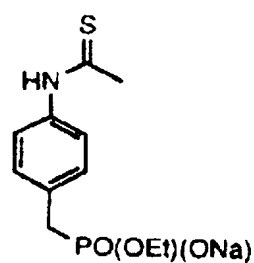
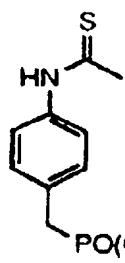
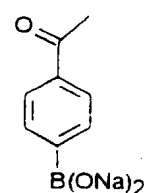
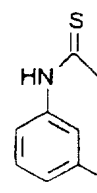
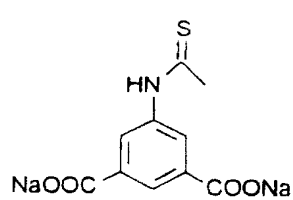
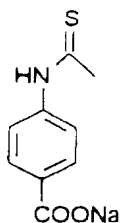
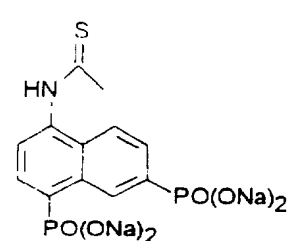
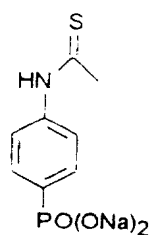
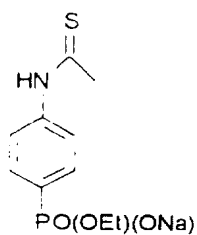
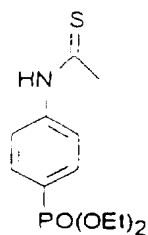
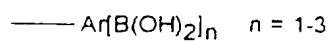
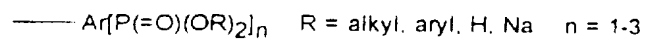
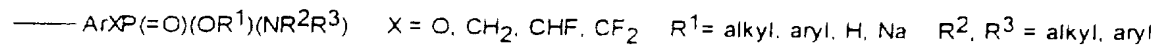
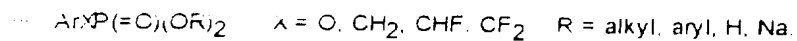
A is an anionic-containing moiety.

3. A compound according to claim 2, wherein said linear polymer has a median range of molecular weight distribution from 1,000 to 1,000,000
4. A compound according to claim 3, wherein said median range of molecular weight distribution is from 10,000 to 600,000.
5. A compound according to claim 2, wherein said monomer unit R is an amine or amide moiety.
6. A compound according to claim 5 wherein said monomer unit R is a lysine or other

7. A compound according to claim 2, wherein said linking group X, when present, is a functional linking group selected from the group consisting of esters, amides, ethers, thioethers, amines, ureas, thioureas, carbamates and carbonates.
8. A compound according to claim 2, wherein said linking group X, when present, is a spacer group selected from the group consisting of alkyl chains which may be optionally substituted or branched; alkoxy, polyalkoxy, alkylthio or polyalkylthio chains which may be optionally substituted; alkenyl, multiple alkenyl, alkynyl or multiple alkynyl chains which may be optionally substituted; and groups of the formula $-(CH_2)_n-2-(CH_2)_n-$ wherein C is $-CH_2-$, $-CH=CH-$, $-C\equiv C-$, $-O-$ or $-S-$, and n is an integer of from 1 to 15.
9. A compound according to any one of claims 1 to 8, wherein said anionic- or cationic-containing moiety or moieties are bonded to amine, sulfhydryl, hydroxy or other reactive functional side chain groups of said linear polymer by amide or thiourea linkages.
10. A compound according to any of claims 1 to 9, wherein said anionic-containing moieties are selected from the group consisting of sulfonic acid-containing moieties, carboxylic acid-containing moieties (including neuraminic and sialic acid-containing moieties and modified neuraminic and sialic acid-containing moieties), boronic acid-containing moieties, and phosphoric and phosphonic acid-containing moieties (including esterified phosphoric and phosphonic acid-containing moieties).
11. A compound according to any one of claims 1 to 9, wherein the moiety or moieties which are bonded to amino or other reactive functional side chain groups are selected from the following groups, in which n is zero or a positive integer:



- 25 -



- 26 -

12. A compound according to claim 1 or claim 2, which is selected from the group consisting of:
 - i. 4-sulfophenylthiourea terminated poly-L-lysines;
 - ii. 3,6-disulfonaphthylthiourea terminated poly-L-lysines;
 - iii. (8-octanamido)-5-acetamido-3,5-dideoxy-2-thio-D-glycero- α -D-galacto-2-nonulopyranosidic acid terminated poly-L-lysines;
 - iv. 3,5-dicarboxyphenylthiourea terminated poly-L-lysines;
 - v. 4-(phosphonomethyl)phenylthiourea terminated poly-L-lysines;
 - vi. 1-phosphonooxyphenyl-4-thiourea terminated poly-L-lysines; and
 - vii. benzamido-4-boronic acid terminated poly-L-lysines.
13. A pharmaceutical or veterinary composition for prophylactic or therapeutic antiviral treatment of a human or non-human animal, which comprises a compound of any of claims 1 to 12, in association with at least one pharmaceutically or veterinarily acceptable carrier or diluent.
14. A method for prophylactic or therapeutic antiviral treatment of a human or non-human animal, which comprises administering to said human or animal or prophylactic- or therapeutic-antiviral-effective amount of a compound of any of claims 1 to 12.
15. A method according to claim 14, wherein said antiviral treatment is treatment of infection by HIV-1 or HIV-2, Hepatitis B or C, Bovine Viral Diarrhoea Virus, Japanese Encephalitis Virus (JEV), Human Influenza Virus A and B, Rhinoviruses, Corona Viruses, Human Parainfluenza Virus, Respiratory Syncytial Virus (RSV), Varicella Zoster Virus (VSV), Human Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Papilloma Virus (HPV), Adenoviruses, Herpes Simplex Virus (HSV) type 1 and 2, Measles Virus or Vesicular Stomatitis Virus (VSV).

- 27 -

16. Use of a compound of any of claims 1 to 12, in the prophylactic or therapeutic antiviral treatment of, or in the manufacture of a medicament for prophylactic or therapeutic antiviral treatment of, a human or non-human animal.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00446

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl⁶: CO8G 69/36, A61K 31/795

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Chemical Abstracts On-line Search, Keywords=(? viral or ? virus) and (poly-L-lysine or polylysine) and (sulf? or phos? or ?ionic or acid)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| X | WO A 95 34595 (Biomolecular Research Institute Ltd), 21 December 1995 (21.12.95). | 1-16 |
| X | AU A 41811/96 (Michel Geffard) 17 June 1996, (17.06.96). See page 17 and the examples of the English translation. | 1-7, 9-11, 13 |
| X | EP A 254 419 (Chisso Corporation), 27 January 1988 (27.01.88). | 1-6, 8, 13 |



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search
1 August 1997 (01.08.97)

Date of mailing of the international search report

27 AUG 1997

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00446

| C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|--|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | Y-W Yang and J-C Yang, "Inhibitory effect of polyionic compounds on the absorbtion of herpes simples virus type 1(KOS)", Antiviral Chemistry and Chemotherapy, 1997, (8(1), 32-37. | 1-16 |
| X | L. Sinibaldi et al., "Effect of Biological and Synthetic Polymers on BK Virus Infectivity and Haemagglutination", Journal of Chemotherapy 4(1), 1992, 16-22. | 1-16 |
| X | R. Roy et al., "Solid Phase Synthesis of Dendritic Dialoside Inhibitors of Influenza A Virus Haemagglutinin", J.C.S., Chem. Commun., 1993, 1869-1872. | 1-16 |
| X | O. Bagasra et al., "Anti-Human Immunodeficiency Virus Type 1 Activity of Sulfated Monosaccharides. Comparison with sulfated Polysaccharides and Other Polyions", The Journal of Infectious Diseases, 1991, 164, 1082-90. | 1-16 |
| X | M. Hosoya et al., "Inhibitory effects of polycations on the replication of enveloped viruses (HIV, HSV, CMV, RSV, influenza A virus and togaviruses) <i>in vitro</i> ", Antiviral Chemistry and Chemotherapy, 1991, 2(4), 243-248. | 1-16 |
| X | K. B. Mathur et al., "Antiviral Activity of Some New Cationic Polyamino Acids", Indian Journal of Experimental Biology, 1982, 20, 227-229. | 1-16 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 97/00446

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | | | | Patent Family Member | | | |
|--|----------|----|-----------|----------------------|-----------|----|-----------|
| WO | 95 34595 | AU | 26659/95 | CA | 2 192 446 | EP | 765 357 |
| AU | 96 41811 | WO | 96 15810 | FR | 2 727 117 | | |
| EP | 254 419 | DE | 3 776 999 | JP | 63 002901 | US | 4 867 974 |
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